CrossMark

Differential abundance of sarcoplasmic proteome explains animal effect on beef *Longissimus lumborum* color stability ☆

Anna C.V.C.S. Canto [a,b], Surendranath P. Suman [a,*], Mahesh N. Nair [a], Shuting Li [a], Gregg Rentfrow [a], Carol M. Beach [c], Teofilo J.P. Silva [b], Tommy L. Wheeler [d], Steven D. Shackelford [d], Adria Grayson [d], Russell O. McKeith [d], D. Andy King [d]

[a] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA
[b] Departamento de Tecnologia de Alimentos, Faculdade de Veterinaria, Universidade Federal Fluminense, Niteroi, 24230-340, Rio de Janeiro, Brazil
[c] Proteomics Core Facility, University of Kentucky, Lexington, KY 40506, USA
[d] USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933, USA

ABSTRACT

The sarcoplasmic proteome of beef *Longissimus lumborum* demonstrating animal-to-animal variation in color stability was examined to correlate proteome profile with color. *Longissimus lumborum* (36 h post-mortem) muscles were obtained from 73 beef carcasses, aged for 13 days, and fabricated to 2.5-cm steaks. One steak was allotted to retail display, and another was immediately vacuum packaged and frozen at −80 °C. Aerobically packaged steaks were stored under display, and color was evaluated on days 0 and 11. The steaks were ranked based on redness and color stability on day 11, and ten color-stable and ten color-labile carcasses were identified. Sarcoplasmic proteome of frozen steaks from the selected carcasses was analyzed. Nine proteins were differentially abundant in color-stable and color-labile steaks. Three glycolytic enzymes (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2) were over-abundant in color-stable steaks and positively correlated ($P < 0.05$) to redness and color stability. These results indicated that animal variations in proteome contribute to differences in beef color.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Color of fresh beef is one of the most important attributes influencing the purchase intention (Mancini & Hunt, 2005; Suman, Hunt, Nair, & Rentfrow, 2014). Consumers prefer a bright cherry-red color for beef and associate it with freshness and wholesomeness. Color deterioration during retail display is perceived as undesirable because discolored meat is often considered unwholesome by consumers, leading to product rejection. In the United States, the beef industry loses more than $1 billion per year due to discoloration (Smith, Belk, Sofos, Tatum, & Williams, 2000). The pigment primarily responsible for meat color is myoglobin; the redox stability of myoglobin and color of fresh meat are governed by a multitude of endogenous as well as exogenous factors (Suman & Joseph, 2013).

Beef color stability is a highly muscle-specific trait (Joseph, Suman, Rentfrow, Li, & Beach, 2012; King, Shackelford, & Wheeler, 2011; McKenna et al., 2005). With respect to the biochemistry of meat color,

Longissimus lumborum (LL) is a major beef muscle that has been extensively studied. LL is a relatively color-stable muscle and demonstrates low oxygen consumption rate (O'Keeffe & Hood, 1982) and increased metmyoglobin reducing activity (Ledward, 1985; Seyfert et al., 2006), both of which favor formation of reduced redox ferrous forms of myoglobin. Previous research in the United States suggested that animal-to-animal variations influence the color stability and discoloration of beef LL steaks during retail display (King, Shackelford, Rodriguez, & Wheeler, 2011; King, Shackelford, & Wheeler, 2011). Nonetheless, the molecular mechanisms of these variations are yet to be completely understood.

The advances in proteomic techniques, such as mass spectrometry, two-dimensional electrophoresis, and bioinformatics, have been applied successfully to explain fundamental bases of meat color phenomena (Suman, Rentfrow, Nair, & Joseph, 2014). Previous research successfully employed proteomic tools to interpret species-specific nature of meat color stability in livestock and poultry (Joseph, Suman, Li, Beach, & Claus, 2010; Nair, Suman, Li, Joseph, & Beach, 2014; Suman, Faustman, Stamer, & Liebler, 2007). Further investigations documented the contribution of sarcoplasmic proteome on muscle-specificity in beef color (Joseph et al., 2012; Suman, Nair et al., 2014). Although a proteomic approach could elucidate the biochemical basis of animal effect on

beef color stability, investigations are yet to be undertaken in this direction. Therefore, the objectives of the present study were — (1) to characterize the sarcoplasmic proteome of LL steaks from beef carcasses demonstrating variations in retail color stability; and (2) to correlate the color stability attributes to differentially abundant proteome components.

2. Materials and methods

2.1. Sample collection

Seventy-three beef carcasses were selected from a commercial processing facility as they were presented for grading at approximately 36 h post-mortem. After ribbing between the 12th and 13th ribs, carcass grade data were collected using an image analysis-based (VBG 2000) grading system (Shackelford, Wheeler, & Koohmaraie, 2003). All carcasses (USDA Select) demonstrated normal lean color and firmness, and had similar marbling scores (between Slight[00] and Slight[90]). In addition, the pH was measured on the anterior surface of the LL muscle on the right side of each carcass with a Reed SD-230 handheld pH meter (Reed Instruments, Wilmington, NC, USA). The carcasses were fabricated, and the striploins (IMPS #180; NAMP, 2007) were obtained from the left sides. Striploins were transported in a refrigerated truck (0 °C) to the U.S. Meat Animal Research Center abattoir and were aged until 13 days post-mortem. After aging, striploins were cut, and the LL muscle was separated. The most anterior third of LL was removed, and the remaining portion was cut into 2.54-cm steaks. One steak was allotted to simulated retail display, and another steak was immediately vacuum packaged and frozen at −80 °C.

2.2. Simulated retail display and instrumental color evaluation

The steaks allotted to retail display were placed on polystyrene trays with soaker pads and over-wrapped with oxygen-permeable polyvinylchloride (PVC) film (stretchable meat film 55003815; Prime Source, St. Louis, MO, USA; oxygen transmission rate = 1.4 mL/cm^2/24 h at 23 °C). Individually packaged steaks were placed under continuous fluorescent lighting (color temperature = 3500 K; color rendering index = 86; 32 W; T8 Ecolux bulb, model number F32T8/SPX35, GE, GE Lighting, Cleveland, OH, USA) for 11 days. Light intensity at the meat surface was approximately 2000 lx. Retail display was conducted in a refrigerated room (1 °C), and no temperature fluctuations associated with defrost cycles were encountered.

Instrumental color readings were taken at two random locations on each steak on days 0 and 11 of retail display. CIE L^* (lightness), a^* (redness), and b^* (yellowness) values were measured on the light-exposed steak surfaces with a HunterLab Miniscan XE Plus colorimeter (Hunter Associates Laboratory, Reston, VA, USA) using 2.54 cm diameter aperture, illuminant A, and 10° standard observer (AMSA, 2012). In addition, the ratio of reflectance at 630 nm and 580 nm (R630/580) was calculated as an indirect estimate of surface color stability; a greater ratio indicates a lesser amount of metmyoglobin/brown discoloration and thus greater color stability. On day 0, steaks were allowed to bloom at least 2 h (after aerobically packaged) in retail display before color evaluation.

Instrumental color data on steaks from the seventy-three carcasses were ranked based on the a^* value and R630/580 on day 11. From this ranking, the ten (n = 10) most color-stable and ten (n = 10) most color-labile steaks were identified to examine the molecular basis of animal-to-animal variation in color stability. The carcasses corresponding to these steaks were identified, and the vacuum-packaged frozen LL steaks from the selected twenty carcasses (collected during fabrication) were shipped in dry ice to the University of Kentucky for proteome analysis.

2.3. Myoglobin concentration

Myoglobin concentration was determined according to the method of Faustman and Phillips (2001). Duplicate 5 g frozen samples were homogenized in 45 mL ice cold 40 mM sodium phosphate buffer at pH 6.8. The homogenate was filtered using Whatman no. 1 filter paper, and the absorbance of the filtrate at 525 nm (A525) was recorded using UV-2401PC spectrophotometer (Shimadzu Inc., Columbia, MD, USA) with sodium phosphate buffer as blank. Myoglobin concentration was calculated using the following equation.

$$\text{Myoglobin (mg/g)} = [\text{A525}/(7.6 \text{ mM}^{-1} \text{ cm}^{-1} \times 1 \text{ cm})] \times [17,000/1000] \times 10$$

where, 7.6 mM^{-1} cm^{-1} = millimolar extinction coefficient of myoglobin at 525 nm; 1 cm = path length of cuvette; 17,000 Da = average molecular mass of myoglobin; 10 = dilution factor.

2.4. Isolation of sarcoplasmic proteome

The sarcoplasmic proteome from beef LL steaks (color-stable and color-labile groups) was extracted as described by Joseph et al. (2012). Frozen samples (5 g) were cut and homogenized in 25 mL ice-cold extraction buffer (40 mM Tris, 2 mM EDTA, and pH 8.0). The homogenate was centrifuged at 10,000 ×g for 15 min at 4 °C. The supernatant consisting of soluble sarcoplasmic proteome was filtered and utilized for analysis.

2.5. Two-dimensional electrophoresis (2-DE)

Bradford assay was used to determine the protein concentration of the sarcoplasmic proteome extract (Bio-Rad, Hercules, CA, USA). The sarcoplasmic protein extract (1200 μg) was mixed with rehydration buffer optimized to 7 M urea, 2 M thiourea, 4% CHAPS, 20 mM DTT, 0.5% Bio-Lyte 5/8 ampholyte (Bio-Rad), and 0.001% bromophenol blue. The mix was applied onto immobilized pH gradient (IPG) strips (pH 5–8, 17 cm), and was subjected to passive rehydration for 16 h. After passive rehydration, the IPG strips were subjected to first-dimension isoelectric focusing (IEF) in a Protean IEF cell system (Bio-Rad) by applying a linear voltage increase initially, and a final rapid voltage ramping to reach a total of 80 kVh. Subsequently the IPG strips were equilibrated in SDS-containing buffers, first with equilibration buffer I (containing 6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% SDS, 20% glycerol, 2% (w/v) DTT; Bio-Rad) followed by equilibration buffer II (containing 6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% SDS, 20% glycerol, 2.5% iodoacetamide; Bio-Rad), each for 15 min. In the second dimension, 13.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 38.5:1 ratio of acrylamide to bis-acrylamide) was employed for protein separation, using Protean II XL system (Bio-Rad). The staining was performed for 48 h using colloidal Coomassie Blue, and the destaining was done for 48 h until the background was cleared. Color-stable and color-labile samples were run in parallel under the same conditions. Two gels per steak were produced; ten steaks were used for each group resulting in 40 gels.

2.6. Gel image analysis

The gel images were digitalized using VersaDoc (Bio-Rad) and were analyzed by PDQuest software (Bio-Rad). Spots were detected and then matched with the aid of landmarks, which are well-resolved spots present in every gel image. Matched spots exhibiting a 1.5-fold or more intensity difference between color-stable and color-labile groups and associated with 5% statistical significance ($P < 0.05$) in the Student's t-test were considered differentially abundant (Joseph et al., 2012).

2.7. Protein identification by mass spectrometry

Pipet tips were used to remove the selected spots from the gels. The spots were destained with 50 mM NH_4HCO_3/50% CH_3CN, homogenized using vortex for 10 min, and dried in a vacuum centrifuge. The proteins present in the gel pieces were first reduced by addition of 10 mM DTT in 50 mM NH_4HCO_3, followed by alkylation with 50 mM iodoacetamide in 50 mM NH_4HCO_3, both for 30 min. The gel pieces were washed two times using 50 mM NH_4HCO_3 solution, once with CH_3CN, and then partially dried. Further, the gel pieces were rehydrated for 1 h (on ice) with 40 mM NH_4HCO_3/9% CH_3CN, containing proteomic grade trypsin (Sigma, St. Louis, MO, USA) at a concentration of 20 ng/μL. Additional volume of 40 mM NH_4HCO_3/9% CH_3CN was added, and the samples were incubated for 18 h at 37 °C. Two extractions of the peptides from the gel pieces were done — first using 0.1% trifluoroacetic acid, and second using a solution of 50% acetonitrile containing 0.1% trifluoroacetic acid; both extracts were then combined. The concentration and desalting of the peptide extracts were performed by solid phase extraction using a 0.1–10 μL pipet tip (Sarstedt, Newton, NC, USA) packed with 1 mm of Empore C-18 (3 M, St. Paul, MN, USA). The peptides were eluted in 5 μL of 50% CH_3CN/0.1% trifluoroacetic acid solution.

The concentrated and desalted peptide extracts (0.3 μL) were transferred onto an Opti-TOF 384 well insert (Applied Biosystems, Foster City, CA, USA) using 0.3 μL of 5 mg/mL α-cyano-4-hydroxycinnamic acid (Aldrich, St. Louis, MO, USA) in 50% CH_3CN/0.1% trifluoroacetic acid. A 4800 MALDI TOF-TOF Proteomics Analyzer (Applied Biosystems) was used to analyze the crystallized samples. From the initial MALDI MS spectrum for each spot, 15 peptide peaks with a signal-to-noise ratio of >20 were subjected to MS–MS for fragmentation and analysis by post-source decay. The MS–MS data were submitted for database similarity search using Protein Pilot 2.0 (Applied Biosystems) in the National Center for Biotechnology Information (NCBI) and UniProt databases to identify proteins.

2.8. Statistical analysis

In this study, LL steaks from ten carcasses were used in each treatment (color-stable and color-labile) providing ten replicates (n = 10). The PROC MIXED procedure (SAS, 2011) with a repeated measure design was used to analyze the data on instrumental color parameters (L^*, a^*, b^*, and R630/580) at 0 and 11 days of retail display. The effects of treatment (color-stable vs. color-labile), retail display, and their interaction were analyzed. Data for muscle pH and myoglobin concentration were analyzed for the effect of treatment. The differences between means were detected using Least Significant Difference (LSD) test at 5% significance ($P < 0.05$) level. In addition, PROC CORR procedure was used to determine the Pearson's correlation coefficients between the differentially abundant protein spots and the instrumental color parameters (SAS, 2011).

3. Results and discussion

3.1. Muscle pH and myoglobin concentration

The pH (measured in the carcass sides) was similar ($P > 0.05$) for the color-stable (5.70 ± 0.04) and color-labile (5.61 ± 0.03) LL muscles. Furthermore, the concentration of myoglobin was not different ($P > 0.05$) between color-stable (4.05 ± 0.27 mg/g) and color-labile (4.56 ± 0.23 mg/g) steaks. In partial agreement with our results, Sammel et al. (2002) observed no difference in the myoglobin concentration of beef inside and outside *Semimembranosus* muscles, which exhibited differences in color stability. In contrast to our findings, King, Shackelford, Rodriguez, and Wheeler (2011) reported greater myoglobin concentration in the color-labile beef loin steaks than in color-stable loin steaks. Several previous investigations attempted to characterize the relationship between myoglobin concentration and color stability in different

beef muscles. McKenna et al. (2005) examined color biochemistry in 19 different beef muscles and reported that, in general, color-stable muscles exhibited lower myoglobin content than the color-labile ones. Furthermore, King, Shackelford, and Wheeler (2011) reported that color-stable beef longissimus muscle exhibited lower myoglobin concentration than the color-labile *Triceps brachii*. Jeong et al. (2009) also reported lower myoglobin concentration in color-stable beef longissimus than in color-labile *Psoas major*.

3.2. Instrumental color

The instrumental color data of color-stable and color-labile steaks on days 0 and 11 of retail display are presented in Table 1. All the color parameters demonstrated a decrease ($P < 0.05$) from day 0 to day 11. While several previous studies investigated intermuscular variation in beef color stability (Joseph et al., 2012; McKenna et al., 2005; Seyfert et al., 2006), limited work has been undertaken to examine color stability variations observed in a specific muscle from different carcasses. In the present study, on day 0, the color-labile steaks demonstrated greater ($P < 0.05$) a^* values (redness) than the color-stable ones; nonetheless a^* values of the two groups were numerically close. On the other hand, the L^* (lightness), b^* (yellowness), and R630/580 were similar ($P > 0.05$) for the two groups. In partial agreement with our data, King, Shackelford, Rodriguez, and Wheeler (2011) studied the color stability of the beef *Longissimus thoracis* and observed greater a^* values in the color-labile steaks than in the color-stable ones on the first day of display, whereas the L^* values and metmyoglobin content on meat surface were not different between the two groups.

After eleven days of refrigerated retail display, the L^* values were similar ($P > 0.05$) for both groups (Table 1). Color-stable steaks exhibited greater ($P < 0.05$) a^*, b^*, and R630/580 than the color-labile samples on day 11. Although both groups demonstrated a decrease ($P < 0.05$) in a^*, b^*, and R630/580, the color-labile steaks exhibited a greater decline between days 0 and 11 than their color-stable counterparts. In agreement with our results, King, Shackelford, Rodriguez, and Wheeler (2011) reported that longissimus steaks in color-stable group exhibited greater a^* and b^* values and lower metmyoglobin content on surface than the color-labile longissimus steaks on day 6 of retail display.

3.3. Sarcoplasmic proteome profile

The image analyses of the Coomassie-stained gels identified twelve differentially abundant protein spots (Fig. 1 and Table 2) in color-stable and color-labile longissimus muscles. Nine protein spots over-abundant ($P < 0.05$) in color-stable steaks (Table 3) were identified as phosphoglucomutase-1 (in 2 different spots), glyceraldehyde-3-phosphate dehydrogenase (in 3 different spots), pyruvate kinase
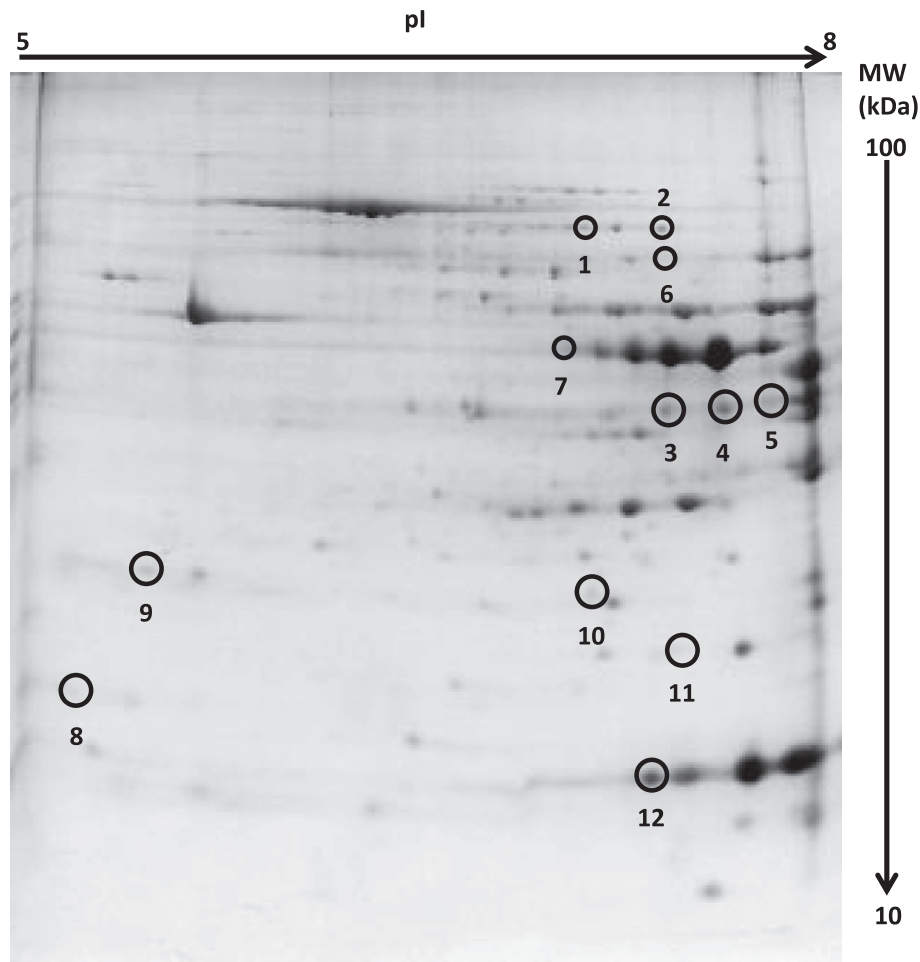
Table 1
Instrumental color of color-stable and color-labile beef *Longissimus lumborum* steaks during refrigerated retail display.

Parameter	Category	Display days	
		0	11
L^* value	Color-stable	42.52 ± 0.83 [ax]	40.48 ± 1.06 [bx]
	Color-labile	43.32 ± 0.93 [ax]	38.79 ± 1.18 [bx]
a^* value	Color-stable	34.65 ± 0.46 [ay]	31.49 ± 0.90 [bx]
	Color-labile	36.43 ± 0.40 [ax]	12.22 ± 0.57 [by]
b^* value	Color-stable	27.12 ± 0.55 [ax]	25.78 ± 0.76 [bx]
	Color-labile	28.65 ± 0.47 [ax]	17.42 ± 0.84 [by]
R630/580	Color-stable	8.71 ± 0.28 [ax]	6.97 ± 0.47 [bx]
	Color-labile	9.45 ± 0.36 [ax]	1.17 ± 0.04 [by]

Results (n = 10) are expressed as the mean ± standard error.
Means without common superscripts (a–b) in a row are different ($P < 0.05$).
Means without common superscripts (x–y) in a column within a parameter are different ($P < 0.05$).

Fig. 1. Coomassie-stained two-dimensional gel of the sarcoplasmic proteome extracted from beef *Longissimus lumborum* steak. Twelve protein spots differentially abundant in color- stable and color-labile steaks are numbered.

M2, creatine kinase M-type, myosin regulatory light chain 2, and myosin light chain 1/3. The other three protein spots were over-abundant ($P < 0.05$) in color-labile samples (Table 3) and were identified as adenylate kinase isoenzyme 1, phosphatidylethanolamine-binding protein 1, and myoglobin. The differentially abundant proteins were involved in glycolysis, ATP metabolism, muscle contraction, signaling, and oxygen transport (Table 3).

3.4. Functional roles of differentially abundant proteins and their correlation with color traits

Six differentially abundant proteins correlated ($P < 0.05$; Table 4) with instrumental color parameters. Five proteins (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase M2, myosin regulatory light chain 2, and myosin light chain 1/3) over-abundant

Table 2
Differentially abundant sarcoplasmic proteins in color-stable and color-labile beef *Longissimus lumborum* steaks.

Spot[a]	Accession number	Protein	Species	ProtScore/ matched peptides	Sequence coverage (%)
1	Q08DP0	Phosphoglucomutase-1	*Bos taurus*	16.43/14	25.4
2	Q08DP0	Phosphoglucomutase-1	*Bos taurus*	22.00/13	30.1
3	P10096	Glyceraldehyde-3-phosphate dehydrogenase	*Bos taurus*	8.40/6	21.9
4	P10096	Glyceraldehyde-3-phosphate dehydrogenase	*Bos taurus*	5.65/4	10.8
5	P10096	Glyceraldehyde-3-phosphate dehydrogenase	*Bos taurus*	7.36/6	17.1
6	gi|73587283	Pyruvate kinase M2	*Bos taurus*	8.19/4	15.4
7	Q9XSC6	Creatine kinase M-type	*Bos taurus*	11.82/9	20.2
8	Q0P571	Myosin regulatory light chain 2	*Bos taurus*	9.52/4	30
9	A0JNJ5	Myosin light chain 1/3	*Bos taurus*	12.00/11	42.7
10	P00570	Adenylate kinase isoenzyme 1	*Bos taurus*	13.03/11	40.2
11	P13696	Phosphatidylethanolamine-binding protein 1	*Bos taurus*	5.36/3	37.4
12	P02192	Myoglobin	*Bos taurus*	12.00/11	48.1

For each spot, parameters related to protein identification are provided, including accession number; species; ProtScore and number of matched peptides; sequence coverage of peptides in tandem mass spectrometry.

[a] Spot number refers to the numbered spots in gel image (Fig. 1).

Table 3
Functional roles of the differentially abundant sarcoplasmic proteins in color-stable and color-labile beef *Longissimus lumborum* steaks.

Spot[a]	Protein	Function	Over-abundant category	Spot ratio
1	Phosphoglucomutase-1	Glycolytic enzyme	Color-stable	1.8[b]
2	Phosphoglucomutase-1	Glycolytic enzyme	Color-stable	2.1[b]
3	Glyceraldehyde-3-phosphate dehydrogenase	Glycolytic enzyme	Color-stable	1.9[b]
4	Glyceraldehyde-3-phosphate dehydrogenase	Glycolytic enzyme	Color-stable	2.0[b]
5	Glyceraldehyde-3-phosphate dehydrogenase	Glycolytic enzyme	Color-stable	2.4[b]
6	Pyruvate kinase M2	Glycolytic enzyme	Color-stable	1.7[b]
7	Creatine kinase M-type	ATP regeneration	Color-stable	1.8[b]
8	Myosin regulatory light chain 2	Muscle contraction	Color-stable	2.4[b]
9	Myosin light chain 1/3	Muscle contraction	Color-stable	2.0[b]
10	Adenylate kinase isoenzyme 1	Adenosine phosphate metabolism	Color-labile	1.6[c]
11	Phosphatidylethanolamine-binding protein 1	Signaling	Color-labile	1.7[c]
12	Myoglobin	Oxygen transport	Color-labile	2.3[c]

[a] Spot number refers to the numbered spots in gel image (Fig. 1).
[b] Spot ratio of color-stable/color-labile.
[c] Spot ratio of color-labile/color-stable.

in color-stable group exhibited a positive correlation ($P < 0.05$) with a^* value (r = 0.52–0.69). In addition, four proteins (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase M2, and myosin regulatory light chain 2) over-abundant in color-stable steaks were positively correlated (r = 0.54–0.65) to R630/580 ($P < 0.05$). On the other hand, phosphatidylethanolamine-binding protein 1, over-abundant in the color-labile group, demonstrated a negative correlation ($P < 0.05$) with a^* value (r = −0.58) and R630/580 (r = −0.59).

3.4.1. Glycolytic enzymes

Three different enzymes (phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase M2) involved in glycolytic metabolism were over-abundant ($P < 0.05$) in the color-stable group (Table 3). The presence of different isoforms of phosphoglucomutase-1 and glyceraldehyde-3-phosphate dehydrogenase exhibiting different isoelectric pH (Fig. 1) could be attributed to possible post-translational modifications such as phosphorylation previously reported in these proteins (Anderson, Lonergan, & Huff-Lonergan, 2014; Bouley, Chambon, & Picard, 2004; Huang et al., 2011).

Phosphoglucomutase-1 catalyzes the reversible transfer of a phosphate group between positions 1 and 6 in a glucose molecule (Cori, Colowick, & Cori, 1938). Phosphorylation of threonine residue at position 466 increases the enzymatic activity of this protein (Gururaj, Barnes, Vadlamudi, & Kumar, 2004) accelerating the conversion of glucose-1-phosphate to glucose-6-phosphate (Anderson et al., 2014), which in turn favors the generation of substrates necessary for regeneration of NADH. Glyceraldehyde-3-phosphate dehydrogenase is an enzyme catalyzing the reversible conversion of glyceraldehyde-3-phosphate and NAD+ to 1,3-bisphosphoglycerate and NADH (Kim & Dang, 2005). The active enzyme is composed of four identical subunits, each of which contains a reactive cysteine residue; the binding of NAD+ at the reactive cysteines activates the enzyme (Harris & Perham, 1965). The mammalian pyruvate kinase M2 is also a homo-

tetrameric glycolytic enzyme (Wooll et al., 2001) catalyzing the dephosphorylation of phosphoenol pyruvate to pyruvate (Ainsworth & Macfarlane, 1973; Mazurek, 2011).

Phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2 were previously identified in proteome profile studies on bovine skeletal muscles (Bouley et al., 2004; Chaze, Bouley, Chambon, Barboiron, & Picard, 2006) and were related with fast-twitch fibers exhibiting high glycolytic activity (Okumura et al., 2005). The over-abundance of these enzymes can result in an increase in glycolytic metabolism and thus can stimulate the production of NADH and pyruvate, the latter of which is a mitochondrial substrate that promotes NADH regeneration (Ramanathan & Mancini, 2010). NADH is an important cofactor in enzymatic and non-enzymatic metmyoglobin reduction (Echevarne, Renerre, & Labas, 1990; Renerre & Labas, 1987). In support, Ramanathan and Mancini (2010) reported that the addition of pyruvate to beef mitochondria regenerated NADH (through tricarboxylic acid cycle) resulting in an electron transport-mediated metmyoglobin reduction. Several previous investigations documented the color-stabilization effect of NADH in beef and model systems. Kim et al. (2006) reported that the addition of NAD+, lactate, and LDH promoted non-enzymatic metmyoglobin reduction in model system possibly due to NADH regeneration. Moreover, in the same study beef LL steaks enhanced with 2.5% of potassium lactate exhibited increased NADH concentration and improved color stability during retail display. Further studies (Kim, Keeton, Smith, Berghman, & Savell, 2009) investigated the differences in color stability among three beef muscles (LL, *Semimembranosus*, and *Psoas major*) and documented that the color-stable LL demonstrated greater a^* values and NADH concentration than the color-labile *Psoas major* during seven days of retail display. Moreover, addition of pyruvate improved the color stability of beef LL steaks (Ramanathan, Mancini, & Dady, 2011) and muscle homogenates (Mohan, Hunt, Barstow, Houser, & Muthukrishnan, 2010). In addition, pyruvate decreased lipid oxidation in ground beef

Table 4
Pearson's correlation between instrumental color parameters on day 11 retail display and differentially abundant sarcoplasmic proteins in beef *Longissimus lumborum* steaks.

Protein	Over-abundant category	Color parameter	Correlation coefficient
Phosphoglucomutase-1	Color-stable	a^* value	+0.57
Glyceraldehyde-3-phosphate dehydrogenase	Color-stable	a^* value	+0.61
Pyruvate kinase M2	Color-stable	a^* value	+0.55
Myosin regulatory light chain 2	Color-stable	a^* value	+0.69
Myosin light chain 1/3	Color-stable	a^* value	+0.52
Phosphatidylethanolamine-binding protein 1	Color-labile	a^* value	−0.58
Phosphoglucomutase-1	Color-stable	R630/580	+0.62
Glyceraldehyde-3-phosphate dehydrogenase	Color-stable	R630/580	+0.65
Pyruvate kinase M2	Color-stable	R630/580	+0.54
Myosin regulatory light chain 2	Color-stable	R630/580	+0.62
Phosphatidylethanolamine-binding protein 1	Color-labile	R630/580	−0.59

(Ramanathan, Mancini, Van Buiten, Suman, & Beach, 2012) and LL steaks (Ramanathan et al., 2011), and thus can minimize discoloration because lipid oxidation accelerates myoglobin oxidation (Faustman, Sun, Mancini, & Suman, 2010; O'Grady, Monahan, & Brunton, 2001).

The greater glycolytic metabolism in color-stable LL steaks indicates a possible low oxygen consumption, which minimizes myoglobin autoxidation resulting in lower metmyoglobin accumulation than in the color-labile steaks (O'Keeffe & Hood, 1982; Renerre & Labas, 1987). Differences in glycolytic metabolism between LL steaks in the two color-stability categories could thus influence the inherent ability to reduce metmyoglobin and minimize discoloration. This in turn is explained by the observed positive correlation ($P < 0.05$) of the three glycolytic enzymes with a^* values and R630/580 (Table 4). In agreement, several studies reported a correlation between glycolytic enzymes and meat color traits. Joseph et al. (2012) investigated the differences in the sarcoplasmic proteome of color-stable (LL) and color-labile (Psoas major) beef muscles and reported that two glycolytic enzymes (β-enolase and triose phosphate isomerase) were over-abundant in LL and that β-enolase was positively correlated with a^* value. Previous investigations on pork quality reported correlation of phosphoglucomutase-1 and glyceraldehyde-3-phosphate dehydrogenase with instrumental color parameters. Zelechowska, Przybylski, Jaworska, and Sante-Lhoutellier (2012) studied the role of sarcoplasmic proteome in color attributes of pork longissimus and documented that phosphoglucomutase-1 was correlated positively to L^* value, whereas glyceraldehyde-3-phosphate dehydrogenase was positively correlated to b^* value. In contrast, Kwasiborski et al. (2008) reported a negative correlation of phosphoglucomutase-1 with a^* values in pork longissimus.

3.4.2. Creatine kinase M-type

Creatine kinase M-type was over-abundant ($P < 0.05$) in the color-stable LL steaks (Table 3). This sarcoplasmic kinase helps maintaining the ATP–ADP equilibrium in post-mortem skeletal muscles by catalyzing the interconversion of ADP and phosphocreatine to ATP and creatine (McLeish & Kenyon, 2005; Wallimann, Wyss, Brdiczka, Nicolay, & Eppenberger, 1992). The anoxia-induced depletion of ATP in post-mortem skeletal muscles leads to utilization of phosphocreatine by the creatine kinase M-type to generate creatine and ATP (Hamm, 1977). The LL muscle is mostly composed of type IIb fast-twitch fibers, which indicates a predominant glycolytic metabolism (Hamelin et al., 2007). Previous reports documented that fast-twitch muscles have greater creatine kinase content (Okumura et al., 2005) and phosphocreatine concentration (Kushmerick, Moerland, & Wiseman, 1992) than the oxidative muscles. On their investigation using in vitro biological model of skeletal muscle, Lawler, Barnes, Wu, Song, and Demaree (2002) reported that creatine exhibited selective antioxidant property through its free radical scavenging ability. In addition, another study in living cells reported antioxidant functions of creatine through scavenging of reactive oxygen and nitrogen species (Sestili et al., 2006), which are associated with oxidative and nitrosative stress (Ryter et al., 2007). Free radicals promote protein oxidation in biological systems (Stadtman & Berlett, 1997) contributing to meat discoloration (Connolly, Brannan, & Decker, 2002; Faustman et al., 2010). The over-abundance of creatine kinase M-type in color-stable steaks can increase the creatine content, which can minimize myoglobin oxidation and improve color stability. In agreement with our results, Joseph et al. (2012) reported greater abundance of creatine kinase M-type in the color-stable beef LL steaks than in color-labile Psoas major steaks and documented a positive correlation between the enzyme and a^* value. Furthermore, in pork Semimembranosus, Sayd et al. (2006) observed an over-abundance of creatine kinase in light muscles than in their dark counterparts. In addition, Kwasiborski et al. (2008) studied the sarcoplasmic proteome of pork longissimus and reported that creatine kinase was positively correlated to a^* value.

3.4.3. Myofibrillar proteins

Two myofibrillar proteins (myosin regulatory light chain 2 and myosin light chain 1/3) were over-abundant ($P < 0.05$) in color-stable LL steaks (Table 3). Previous research reported that LL muscle is composed predominantly of fast-twitch type IIb fibers (Hamelin et al., 2007; Hwang, Kim, Jeong, Hur, & Joo, 2010), which are associated with glycolytic metabolism (Peter, Barnard, Edgerton, Gillespie, & Stempel, 1972). A myosin molecule consists of two heavy chains, two essential light chains, and two regulatory light chains (Schiaffino & Reggiani, 1996). The two types of essential light chains (myosin light chains 1 and 3) are transcribed from the same gene and thus exhibit significant similarities in their amino acid sequences (Barton & Buckingham, 1985). In skeletal muscles, fast-twitch fibers are mainly composed of fast-type myosin regulatory light chain 2 and myosin light chain 1/3 (Bicer & Reiser, 2004; Schiaffino & Reggiani, 1994). The appearance of myofibrillar proteins in sarcoplasmic fraction can be attributed to the 13 days aging prior to retail display. Previous studies (Lametsch, Roepstorff, & Bendixen, 2002; Lametsch et al., 2006) reported that the cleavage at myosin neck region during aging releases the myosin light chains from actomyosin complex resulting in their migration from myofibrillar proteome to the soluble sarcoplasmic proteome. The myofibrillar proteins over-abundant in color-stable steaks are fast-type indicating predominance of fast-twitch type IIb fibers in color-stable steaks than in color-labile ones. Fast-twitch type IIb fibers are strongly glycolytic (Peter et al., 1972), and the beef muscles demonstrating predominantly glycolytic metabolism are color-stable (O'Keeffe & Hood, 1982).

Myosin regulatory light chain 2 and myosin light chain 1/3 were positively correlated ($P < 0.05$) to a^* values (Table 4). Furthermore, myosin regulatory light chain 2 was positively correlated ($P < 0.05$) to R630/580 (Table 4). Our findings are in partial agreement with those of Oe et al. (2011), who investigated the proteome differences between Masseter (slow-twitch) and Semitendinosus (fast-twitch) muscles from Holstein cows. These authors observed greater abundance of the three proteins of our interest (myosin light chain 1 fast, myosin light chain 3 fast, and myosin regulatory light chain fast) in Semitendinosus than in Masseter. In addition, Semitendinosus demonstrated greater levels of glycolytic enzymes (enolase-3, aldolase-A, glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase) than masseter indicating that fast-twitch muscles are associated with glycolytic metabolism.

3.4.4. Adenylate kinase isoenzyme 1

Adenylate kinase isoenzyme 1 catalyzes the reversible conversion of two molecules of ADP to ATP and AMP (Heil et al., 1974) and was over-abundant ($P < 0.05$) in the color-labile LL steaks (Table 3). While the exact mechanisms through which adenylate kinase influences color stability is not clear, our results are indirectly supported by muscle proteomic studies in Japanese Black cattle (Shibata et al., 2009). These authors investigated the differences in proteome profile of Semitendinosus muscles from grass-fed and grain-fed cattle and reported that adenylate kinase 1 was over-abundant in the grass-fed animals. In addition, the muscles from grass-fed animals exhibited a greater content of slow-twitch myofibrillar proteins suggesting greater proportion of slow-twitch oxidative muscle fibers (Peter et al., 1972) than their counterparts from grain-fed cattle. On the other hand, several glycolytic enzymes (β-enolase 3, fructose-1,6-bisphosphate aldolase A, and triosephosphate isomerase) were over-abundant in the grain-fed cattle indicating predominance of fast-twitch glycolytic muscle fibers. In general, muscles with increased oxidative metabolism demonstrate high oxygen consumption and are color-labile (O'Keeffe & Hood, 1982). Nonetheless, color attributes were not evaluated in the aforementioned study (Shibata et al., 2009) to assess the relationship between muscle proteome and color stability. In contrast, previous proteomic investigations in pork longissimus (Hwang, Park, Kim, Cho, & Lee, 2005; Kwasiborski et al., 2008) reported no correlation between adenylate kinase and color parameters.

3.4.5. Phosphatidylethanolamine-binding protein 1

Phosphatidylethanolamine-binding protein 1 was over-abundant ($P < 0.05$) in color-labile LL steaks (Table 3). This protein, also known as Raf kinase inhibitor protein (Yeung et al., 1999), belongs to phosphatidylethanolamine-binding protein family and is critically involved in cell signaling pathways (Keller, Fu, & Brennan, 2004). This is a cytosolic basic protein demonstrating affinity to organic anions and was named due to its capacity to bind with phosphatidylethanolamine (Bernier & Jolles, 1984; Bernier, Tresca, & Jolles, 1986). Further studies suggested that phosphatidylethanolamine-binding protein has a nucleotide-binding site (Schoentgen et al., 1992) and exhibits affinity to nucleotides such as ATP (Bucquoy, Jolles, & Schoentgen, 1994).

While phosphatidylethanolamine-binding protein 1 demonstrated a negative correlation ($P < 0.05$) with a^* value and R630/580 (Table 4) in the present study, the exact mechanisms through which it influences color biochemistry are not clear. Nonetheless, findings from previous muscle proteomic studies were in agreement with our results. Kwasiborski et al. (2008) observed that this protein was negatively correlated to a^* and L^* values in pork longissimus. Moreover, Shibata et al. (2009) reported an over-abundance of phosphatidylethanolamine-binding protein in the *Semitendinosus* muscles from grass-fed beef cattle compared with their counterparts from grain-fed animals; these authors also observed that the *Semitendinosus* muscles from grass-fed animals exhibited predominance of slow-twitch fibers. Skeletal muscles consisting predominantly of slow-twitch fiber types are oxidative in metabolism (Peter et al., 1972) and thus are color-labile (O'Keeffe & Hood, 1982). Results of the aforementioned studies indicated the necessity of further research on the role of phosphatidylethanolamine-binding protein 1 in meat color.

3.4.6. Myoglobin

Spot 12 (Fig. 1) was over-abundant ($P < 0.05$) in color-labile steaks, and the protein in this spot was identified as myoglobin (Tables 2 and 3). However, myoglobin concentration was similar ($P > 0.05$) in color-stable and the color-labile LL steaks. Therefore, three more protein spots exhibiting molecular weights similar to that of spot 12, but with different isoelectric points (Fig. 2), were subjected to tryptic digestion and tandem mass spectrometry; these three spots were also identified as myoglobin (Table 5). Appearance of four spots identified as myoglobin suggested the possibility of post-translational modifications (Farley & Link, 2009). Post-translational modification of proteins via phosphorylation leads to an acidic shift in the isoelectric pH (Maurides, Akkaraju, & Jagus, 1989; Zhu, Zhao, Lubman, Miller, & Barder, 2005) as observed in Fig. 2. Nonetheless, we did not confirm phosphorylation of myoglobin. The spot over-abundant in color-labile steaks (spot 12) exhibited the most acidic isoelectric point (Fig. 2) insinuating that myoglobin may be post-translationally modified at a greater degree in color-labile steaks than in color-stable ones and that this modification may compromise color stability. While previous studies have reported carbonylation of beef myoglobin (Alderton, Faustman, Liebler, & Hill, 2003; Suman et al., 2007), phosphorylation of myoglobin is yet to be reported in meat-producing livestock. Further research is necessary to examine the possibility of myoglobin phosphorylation and its implication in meat color stability.
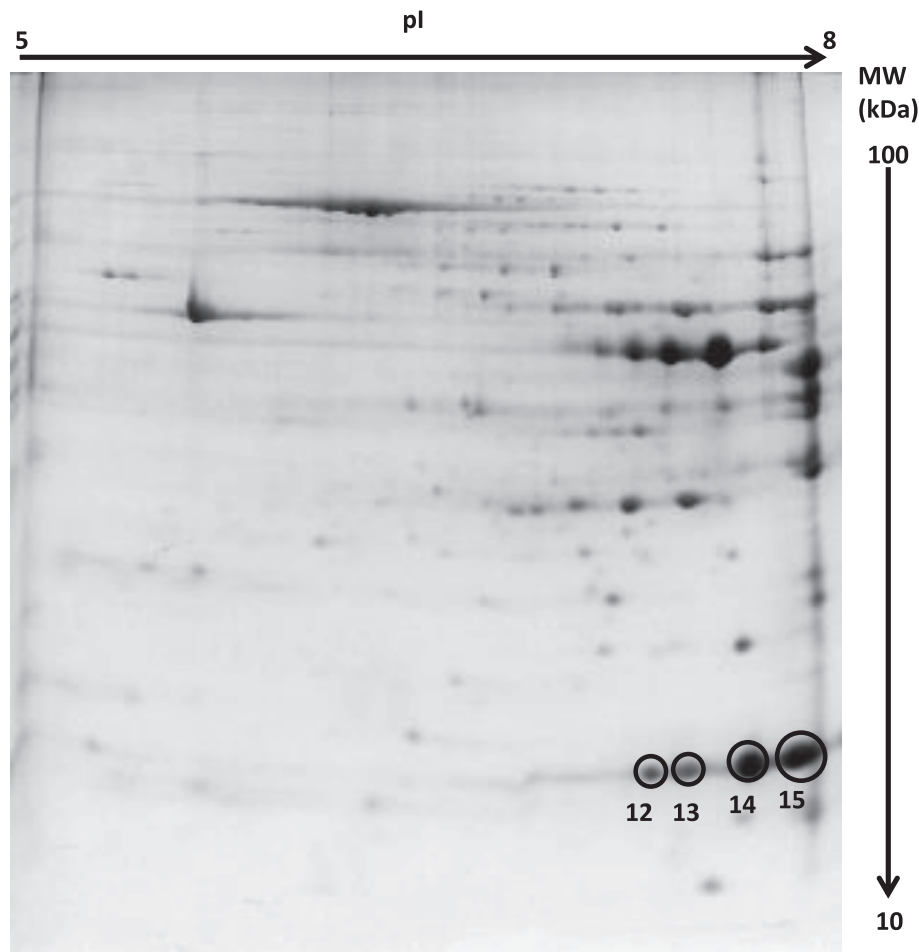

Fig. 2. Coomassie-stained two-dimensional gel of the sarcoplasmic proteome extracted from beef *Longissimus lumborum* steak. Four myoglobin spots detected in color-stable and color-labile steaks are numbered.

Table 5

Myoglobin spots identified in the sarcoplasmic proteome of color-stable and color-labile beef *Longissimus lumborum* steaks.

Spot[a]	Accession no.	Protein	Species	ProtScore/matched peptides	Sequence coverage (%)
12	P02192	Myoglobin	*Bos taurus*	12.00/11	48.1
13	P02192	Myoglobin	*Bos taurus*	9.49/7	48.1
14	P02192	Myoglobin	*Bos taurus*	13.77/13	58.4
15	P02192	Myoglobin	*Bos taurus*	14.00/13	58.4

For each spot, parameters related to protein identification are provided, including accession number; species; ProtScore and number of matched peptides; sequence coverage of peptides in tandem mass spectrometry.

[a] Spot number refers to the numbered spots in gel image (Fig. 2).

Phosphoproteomics is an emerging area in life sciences (Mayya & Han, 2009), and recently an attempt was made to evaluate the relationship between protein phosphorylation and beef tenderness. Anderson et al. (2014) examined the role of phosphorylation in tenderness of beef longissimus and reported that the least phosphorylated isoform of phosphoglucomutase enzyme was over-abundant in the less tender beef samples. These findings indicated the potential of protein post-translational modifications as biomarkers for meat quality.

4. Conclusions

The results of the present study indicate that the animal-to-animal variations observed in beef LL color stability during retail display could be attributed to the differences in sarcoplasmic proteome profile. The over-abundance of glycolytic enzymes in the color-stable LL steaks contributes to improved color stability possibly through NADH regeneration in post-mortem muscles. In addition, possible in situ post-translational modification of myoglobin in color-labile LL steaks appeared to compromise color stability. Further studies should examine the roles of post-translational modifications of myoglobin as well as the interactions between genome and muscle proteome in beef color stability so that biomarkers can be identified for this economically important quality attribute.

Conflict of interest

There are no conflicts of interest. Mention of trade names, proprietary products, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. The USDA is an equal opportunity provider and employer.

References

Ainsworth, S., & Macfarlane, N. (1973). A kinetic study of rabbit muscle pyruvate kinase. *Biochemical Journal, 131,* 223–236.

Alderton, A. L., Faustman, C., Liebler, D. C., & Hill, D. W. (2003). Induction of redox instability of bovine myoglobin by adduction with 4-hydroxy-2-nonenal. *Biochemistry, 42,* 4398–4405.

AMSA (2012). *Meat color measurement guidelines.* Champaign, IL, USA: American Meat Science Association.

Anderson, M. J., Lonergan, S. M., & Huff-Lonergan, E. (2014). Differences in phosphorylation of phosphoglucomutase 1 in beef steaks from the longissimus dorsi with high or low star probe values. *Meat Science, 96,* 379–384.

Barton, P. J., & Buckingham, M. E. (1985). The myosin alkali light chain proteins and their genes. *Biochemical Journal, 231,* 249–261.

Bernier, I., & Jolles, P. (1984). Purification and characterization of a basic 23 kDa cytosolic protein from bovine brain. *Biochimica et Biophysica Acta, 790,* 174–181.

Bernier, I., Tresca, J. P., & Jolles, P. (1986). Ligand-binding studies with a 23 kDa protein purified from bovine brain cytosol. *Biochimica et Biophysica Acta, 871,* 19–23.

Bicer, S., & Reiser, P. J. (2004). Myosin light chain isoform expression among single mammalian skeletal muscle fibers: Species variations. *Journal of Muscle Research and Cell Motility, 25,* 623–633.

Bouley, J., Chambon, C., & Picard, B. (2004). Mapping of bovine skeletal muscle proteins using two-dimensional gel electrophoresis and mass spectrometry. *Proteomics, 4,* 1811–1824.

Bucquoy, S., Jolles, P., & Schoentgen, F. (1994). Relationships between molecular interactions (nucleotides, lipids and proteins) and structural features of the bovine brain 21-kDa protein. *European Journal of Biochemistry, 225,* 1203–1210.

Chaze, T., Bouley, J., Chambon, C., Barboiron, C., & Picard, B. (2006). Mapping of alkaline proteins in bovine skeletal muscle. *Proteomics, 6,* 2571–2575.

Connolly, B. J., Brannan, R. G., & Decker, E. A. (2002). Potential of peroxynitrite to alter the color of myoglobin in muscle foods. *Journal of Agricultural and Food Chemistry, 50,* 5220–5223.

Cori, G. T., Colowick, S. P., & Cori, C. F. (1938). The formation of glucose-1-phosphoric acid in extracts of mammalian tissues and of yeast. *Journal of Biological Chemistry, 123,* 375–380.

Echevarne, C., Renerre, M., & Labas, R. (1990). Metmyoglobin reductase activity in bovine muscles. *Meat Science, 27,* 161–172.

Farley, A. R., & Link, A. J. (2009). Identification and quantification of protein posttranslational modifications. *Methods in Enzymology, 463,* 725–763.

Faustman, C., & Phillips, A. L. (2001). Measurement of discoloration in fresh meat. Ch. F3 Unit F3.3. *Current Protocol in Food Analytical Chemistry.* New York: Wiley and Sons.

Faustman, C., Sun, Q., Mancini, R., & Suman, S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science, 86,* 86–94.

Gururaj, A., Barnes, C. J., Vadlamudi, R. K., & Kumar, R. (2004). Regulation of phosphoglucomutase 1 phosphorylation and activity by a signaling kinase. *Oncogene, 23,* 8118–8127.

Hamelin, M., Sayd, T., Chambon, C., Bouix, J., Bibe, B., Milenkovic, D., Leveziel, H., Georges, M., Clop, A., Marinova, P., & Laville, E. (2007). Differential expression of sarcoplasmic proteins in four heterogeneous ovine skeletal muscles. *Proteomics, 7,* 271–280.

Hamm, R. (1977). Postmortem breakdown of ATP and glycogen in ground muscle: A review. *Meat Science, 1,* 15–39.

Harris, J. l., & Perham, R. N. (1965). Glyceraldehyde 3-phosphate dehydrogenases: I. The protein chains in glyceraldehyde 3-phosphate dehydrogenase from pig muscle. *Journal of Molecular Biology, 13.*

Heil, A., Muller, G., Noda, L., Pinder, T., Schirmer, H., Schirmer, I., & von Zabern, I. (1974). The amino-acid sequence of porcine adenylate kinase from skeletal muscle. *European Journal of Biochemistry, 43,* 131–144.

Huang, H., Larsen, M. R., Karlsson, A. H., Pomponio, L., Costa, L. N., & Lametsch, R. (2011). Gel-based phosphoproteomics analysis of sarcoplasmic proteins in post-mortem porcine muscle with pH decline rate and time differences. *Proteomics, 11,* 4063–4076.

Hwang, Y. H., Kim, G. D., Jeong, J. Y., Hur, S. J., & Joo, S. T. (2010). The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers. *Meat Science, 86,* 456–461.

Hwang, I. H., Park, B. Y., Kim, J. H., Cho, S. H., & Lee, J. M. (2005). Assessment of postmortem proteolysis by gel-based proteome analysis and its relationship to meat quality traits in pig longissimus. *Meat Science, 69,* 79–91.

Jeong, J. Y., Hur, S. J., Yang, H. S., Moon, S. H., Hwang, Y. H., Park, G. B., & Joo, S. T. (2009). Discoloration characteristics of 3 major muscles from cattle during cold storage. *Journal of Food Science, 74,* C1–C5.

Joseph, P., Suman, S. P., Li, S., Beach, C. M., & Claus, J. R. (2010). Mass spectrometric characterization and thermostability of turkey myoglobin. *LWT — Food Science and Technology, 43,* 273–278.

Joseph, P., Suman, S. P., Rentfrow, G., Li, S., & Beach, C. M. (2012). Proteomics of muscle-specific beef color stability. *Journal of Agricultural and Food Chemistry, 60,* 3196–3203.

Keller, E. T., Fu, Z., & Brennan, M. (2004). The role of Raf kinase inhibitor protein (RKIP) in health and disease. *Biochemical Pharmacology, 68,* 1049–1053.

Kim, J. W., & Dang, C. V. (2005). Multifaceted roles of glycolytic enzymes. *Trends in Biochemical Sciences, 30,* 142–150.

Kim, Y. H., Hunt, M. C., Mancini, R. A., Seyfert, M., Loughin, T. M., Kropf, D. H., & Smith, J. S. (2006). Mechanism for lactate-color stabilization in injection-enhanced beef. *Journal of Agricultural and Food Chemistry*, *54*, 7856–7862.

Kim, Y. H., Keeton, J. T., Smith, S. B., Berghman, L. R., & Savell, J. W. (2009). Role of lactate dehydrogenase in metmyoglobin reduction and color stability of different bovine muscles. *Meat Science*, *83*, 376–382.

King, D. A., Shackelford, S. D., Rodriguez, A. B., & Wheeler, T. L. (2011). Effect of time of measurement on the relationship between metmyoglobin reducing activity and oxygen consumption to instrumental measures of beef longissimus color stability. *Meat Science*, *87*, 26–32.

King, D. A., Shackelford, S. D., & Wheeler, T. L. (2011). Relative contributions of animal and muscle effects to variation in beef lean color stability. *Journal of Animal Science*, *89*, 1434–1451.

Kushmerick, M. J., Moerland, T. S., & Wiseman, R. W. (1992). Mammalian skeletal muscle fibers distinguished by contents of phosphocreatine, ATP, and Pi. *Proceedings of National Academy of Sciences of the United States of America*, *89*.

Kwasiborski, A., Sayd, T., Chambon, C., Sante-Lhoutellier, V., Rocha, D., & Terlouw, C. (2008). Pig Longissimus lumborum proteome: Part II: Relationships between protein content and meat quality. *Meat Science*, *80*, 982–996.

Lametsch, R., Kristensen, L., Larsen, M. R., Therkildsen, M., Oksbjerg, N., & Ertbjerg, P. (2006). Changes in the muscle proteome after compensatory growth in pigs. *Journal of Animal Science*, *84*, 918–924.

Lametsch, R., Roepstorff, P., & Bendixen, E. (2002). Identification of protein degradation during post-mortem storage of pig meat. *Journal of Agricultural and Food Chemistry*, *50*, 5508–5512.

Lawler, J. M., Barnes, W. S., Wu, G., Song, W., & Demaree, S. (2002). Direct antioxidant properties of creatine. *Biochemical and Biophysical Research Communications*, *290*, 47–52.

Ledward, D. A. (1985). Post-slaughter influences on the formation of metmyoglobin in beef muscles. *Meat Science*, *15*, 149–171.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science*, *71*, 100–121.

Maurides, P. A., Akkaraju, G. R., & Jagus, R. (1989). Evaluation of protein phosphorylation state by a combination of vertical slab gel isoelectric focusing and immunoblotting. *Analytical Biochemistry*, *183*, 144–151.

Mayya, V., & Han, D. K. (2009). Phosphoproteomics by mass spectrometry: Insights, implications, applications, and limitations. *Expert Review of Proteomics*, *6*, 605–618.

Mazurek, S. (2011). Pyruvate kinase type M2: A key regulator of the metabolic budget system in tumor cells. *The International Journal of Biochemistry & Cell Biology*, *43*, 969–980.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W., & Savell, J. W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science*, *70*, 665–682.

McLeish, M. J., & Kenyon, G. L. (2005). Relating structure to mechanism in creatine kinase. *Critical Reviews in Biochemistry and Molecular Biology*, *40*, 1–20.

Mohan, A., Hunt, M. C., Barstow, T. J., Houser, T. A., & Muthukrishnan, S. (2010). Effects of malate, lactate, and pyruvate on myoglobin redox stability in homogenates of three bovine muscles. *Meat Science*, *86*, 304–310.

Nair, M. N., Suman, S. P., Li, S., Joseph, P., & Beach, C. M. (2014). Lipid oxidation-induced oxidation in emu and ostrich myoglobins. *Meat Science*, *96*, 984–993.

NAMP (2007). *The meat buyer's guide.* Reston, VA: North American Meat Processors Association.

O'Grady, M. N., Monahan, F. J., & Brunton, N. P. (2001). Oxymyoglobin oxidation and lipid oxidation in bovine muscle—mechanistic studies. *Journal of Food Science*, *66*, 386–392.

Oe, M., Ohnishi-Kameyama, M., Nakajima, I., Muroya, S., Shibata, M., Ojima, K., Kushibiki, S., & Chikuni, K. (2011). Proteome analysis of whole and water-soluble proteins in masseter and semitendinosus muscles of Holstein cows. *Animal Science Journal*, *82*, 181–186.

O'Keeffe, M., & Hood, D. E. (1982). Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability. *Meat Science*, *7*, 209–228.

Okumura, N., Hashida-Okumura, A., Kita, K., Matsubae, M., Matsubara, T., Takao, T., & Nagai, K. (2005). Proteomic analysis of slow- and fast-twitch skeletal muscles. *Proteomics*, *5*, 2896–2906.

Peter, J. B., Barnard, R. J., Edgerton, V. R., Gillespie, C. A., & Stempel, K. E. (1972). Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry*, *11*, 2627–2633.

Ramanathan, R., & Mancini, R. A. (2010). Effects of pyruvate on bovine heart mitochondria-mediated metmyoglobin reduction. *Meat Science*, *86*, 738–741.

Ramanathan, R., Mancini, R. A., & Dady, G. A. (2011). Effects of pyruvate, succinate, and lactate enhancement on beef *longissimus* raw color. *Meat Science*, *88*, 424–428.

Ramanathan, R., Mancini, R. A., Van Buiten, C. B. V., Suman, S. P., & Beach, C. M. (2012). Effects of pyruvate on lipid oxidation and ground beef color. *Journal of Food Science*, *77*, C886–C892.

Renerre, M., & Labas, R. (1987). Biochemical factors influencing metmyoglobin formation in beef muscles. *Meat Science*, *19*, 151–165.

Ryter, S. W., Kim, H. P., Hoetzel, A., Park, J. W., Nakahira, K., Wang, X., & Choi, A. M. K. (2007). Mechanisms of cell death in oxidative stress. *Antioxidants & Redox Signaling*, *9*, 49–89.

Sammel, L. M., Hunt, M. C., Kropf, D. H., Hachmeister, K. A., Kastner, C. L., & Johnson, D. E. (2002). Influence of chemical characteristics of beef inside and outside semimembranosus on color traits. *Journal of Food Science*, *67*, 1323–1330.

SAS (2011). *SAS System for Windows. Release 9.3.* Cary, NC, USA: SAS Institute Inc.

Sayd, T., Morzel, M., Chambon, C., Franck, M., Figwer, P., Larzul, C., Le Roy, P., Monin, G., Cherel, P., & Laville, E. (2006). Proteome analysis of the sarcoplasmic fraction of pig *semimembranosus* muscle: Implications on meat color development. *Journal of Agricultural and Food Chemistry*, *54*, 2732–2737.

Schiaffino, S., & Reggiani, C. (1994). Myosin isoforms in mammalian skeletal muscle. *Journal of Applied Physiology*, *77*, 493–501.

Schiaffino, S., & Reggiani, C. (1996). Molecular diversity of myofibrillar proteins: Gene regulation and functional significance. *Physiological Reviews*, *76*, 371–423.

Schoentgen, F., Seddiqi, N., Bucquoy, S., Jolles, P., Lemesle-Varloot, L., Provost, K., & Mornon, J. P. (1992). Main structural and functional features of the basic cytosolic bovine 21 kDa protein delineated through hydrophobic cluster analysis and molecular modelling. *Protein Engineering*, *5*, 295–303.

Sestili, P., Martinelli, C., Bravi, G., Piccoli, G., Curci, R., Battistelli, M., Falcieri, E., Agostini, D., Gioacchini, A. M., & Stocchi, V. (2006). Creatine supplementation affords cytoprotection in oxidatively injured cultured mammalian cells via direct antioxidant activity. *Free Radical Biology & Medicine*, *40*, 837–849.

Seyfert, M., Mancini, R. A., Hunt, M. C., Tang, J., Faustman, C., & Garcia, M. (2006). Color stability, reducing activity, and cytochrome c oxidase activity of five bovine muscles. *Journal of Agricultural and Food Chemistry*, *54*, 8919–8925.

Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2003). On-line prediction of yield grade, longissimus muscle area, preliminary yield grade, adjusted preliminary yield grade, and marbling score using the MARC beef carcass image analysis system. *Journal of Animal Science*, *81*, 150–155.

Shibata, M., Matsumoto, K., Oe, M., Ohnishi-Kameyama, M., Ojima, K., Nakajima, I., Muroya, S., & Chikuni, K. (2009). Differential expression of the skeletal muscle proteome in grazed cattle. *Journal of Animal Science*, *87*, 2700–2708.

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D., & Williams, S. N. (2000). Economic implications of improved color stability in beef. In E. A. Decker, C. Faustman, & C. J. Lopez-Bote (Eds.), *Antioxidants in muscle foods: Nutritional strategies to improve quality* (pp. 397–426). New York: Wiley-Interscience.

Stadtman, E. R., & Berlett, B. S. (1997). Reactive oxygen-mediated protein oxidation in aging and disease. *Chemical Research in Toxicology*, *10*, 485–494.

Suman, S. P., Faustman, C., Stamer, S. L., & Liebler, D. C. (2007). Proteomics of lipid oxidation-induced oxidation of porcine and bovine oxymyoglobins. *Proteomics*, *7*, 628–640.

Suman, S. P., Hunt, M. C., Nair, M. N., & Rentfrow, G. (2014). Improving beef color stability: Practical strategies and underlying mechanisms. *Meat Science*, *98*, 490–504.

Suman, S. P., & Joseph, P. (2013). Myoglobin chemistry and meat color. *Annual Review of Food Science and Technology*, *4*, 79–99.

Suman, S. P., Nair, M. N., Chatli, M. K., Li, S., Joseph, P., Beach, C. M., & Rentfrow, G. (2014). Differential abundance of sarcoplasmic proteome in beef inside and outside semimembranosus muscle. *Meat Science*, *96*, 490–491.

Suman, S. P., Rentfrow, G., Nair, M. N., & Joseph, P. (2014). Proteomics of muscle- and species-specificity in meat color stability. *Journal of Animal Science*, *92*, 875–882.

Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., & Eppenberger, H. M. (1992). Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: The 'phosphocreatine circuit' for cellular energy homeostasis. *Biochemical Journal*, *281*, 21–40.

Wooll, J. O., Friesen, R. H. E., White, M. A., Watowich, S. J., Fox, R. O., Lee, J. C., & Czerwinski, E. W. (2001). Structural and functional linkages between subunit interfaces in mammalian pyruvate kinase. *Journal of Molecular Biology*, *312*, 525–540.

Yeung, K., Seitz, T., Li, S., Janosch, P., McFerran, B., Kaiser, C., Fee, F., Katsanakis, K. D., Rose, D. W., Mischak, H., Sedivy, J. M., & Kolch, W. (1999). Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. *Nature*, *401*, 173–177.

Zelechowska, E., Przybylski, W., Jaworska, D., & Sante-Lhoutellier, V. (2012). Technological and sensory pork quality in relation to muscle and drip loss protein profiles. *European Food Research and Technology*, *234*, 883–894.

Zhu, K., Zhao, J., Lubman, D. M., Miller, F. R., & Barder, T. J. (2005). Protein pI shifts due to posttranslational modifications in the separation and characterization of proteins. *Analytical Chemistry*, *77*, 2745–2755.